Supporting information

Ultrasound-Responsive Glycopolymer Micelles for Targeted Dual Drug Delivery in Cancer Therapy

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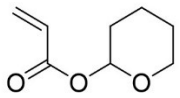
Supplementary Materials

p-Toluenesulfonic acid monohydrate, pyridine, Dihydropyran, pyridine, HATU and Acrylyl chloride were purchased from Aladdin (China). Acrylic acid, Trifluoroacetic acid, dasatinib and Triethylamine were purchased from Energy Chemical (China). Dicyclohexylcarbodiimide and N-Boc-piperazine were purchased from Bide (China). 4-Dimethylaminopyridine, Eosin Y water soluble, DIPEA and Copper sulfate pentahydrate were purchased from J&K Scientific (China). BAPO-ONa has been synthesized[1]. Potassium azide was purchased from Macklin (China). Mono-Propargylamine was purchased from TOKYO Chemical Industry (Japan). Silica was purchased from Sinopharm (China). Antibodies used for immunoblotting assays were purchased from Cell Signalling Technology. CCK-8 assay kit was purchased from Boster Biological Technology (China). Dialysis membrane (molecular weight cut off 2,000 Da) was purchased from 3M Science. Hoechst 33342 was purchased from Maokang Biotechnology Co., Ltd. (Shanghai, China) and used as received. LysoTrackerRed DND-99, Fetal bovine serum (FBS), penicillin, streptomycin, and Dul-becco's Modified Eagle's Medium (DMEM) were purchased from Thermo Fisher Scientific (Waltham, MA, USA) and used as received. Hoechst 33342 was purchased from Maokang Biotechnology Co., Ltd. (Shanghai, China) and used as received. The human liver cancer cell line HepG2 was purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China).

^{1}H NMR spectra were recorded on a Bruker AVA400 spectrometer at 298 K in deuterated solvents. Flow cytometry analysis was carried out on a CytoFLEX V0-B3-R1 Flow Cytometer (Beckman Coulter). The reaction of free radical photopolymerization was used by 365 nm UV curing lights (48 W, HAOSIFA, China). UV-*vis* spectra were recorded using a UV-1800 spectrometer using a quartz cuvette (Shimadzu). The absorbance of 96-well plates was read on a Multiskan Go multimode reader (Thermo Scientific). Confocal images were taken on an Olympus FV1200 confocal microscope. In vivo fluorescent images were taken using an IVIS Spectrum CT system. Ultrasound irradiation were

performed using ultrasound therapy instruments (Haphel, XK-2011T), all-digital ultrasound therapy device (WELLD, 301) was used for ultrasound irradiation in vivo study.

Synthesis of THPA

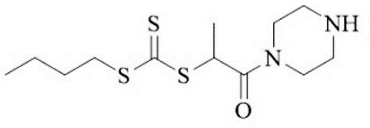


1

Compound 1 (THPA) was synthesized following a reported procedure[2]. *p*-Toluenesulfonic acid monohydrate (0.95 g, 5.00 mmol), pyridine (0.40 mL, 5.00 mmol), and acrylic acid (8.60 g, 119.34 mmol) were dissolved in 100 mL of dichloromethane. Dihydropyran (18.48 mL, 203.66 mmol) was slowly added at room temperature and the reaction was stirred for 24 h. The solution was extracted by water twice to remove the salt, and then extracted by $NaHCO_3$ and NaCl twice respectively. Finally, the solvent was removed under reduced pressure at 35 ℃. The isolated monomer was analysed by ^{1}H NMR. Yield: 60%.

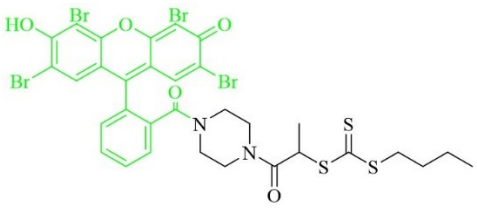
Compound 1, ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.45 - 6.31 (m, 1H), 6.08 (dd, J = 17.2, 10.4 Hz, 1H), 6.02-5.94 (m, 1H), 5.85 - 5.75 (m, 1H), 3.91-3.73 (m, 1H), 3.63 (m, 1H), 1.83 - 1.44 (m, 7H).

Synthesis of Eosin Y-RAFT



2

Synthesis of compound 2. 2-butylsulfanylcarbothioylsulfanylpropanoic acid (0.24 g, 1.00 mmol) was dissolved in dichloromethane and then the solution was cooled to 0℃. Dicyclohexylcarbodiimide (0.23 g, 1.10 mmol) and 10 mL dichloromethane were added in portions with stirring for 10 min. Finally, N-Boc-piperazine (0.25 g, 1.00 mmol), 4-Dimethylaminopyridine (0.02 g, 0.20 mmol) were added to the above reaction solution with stirring at room temperature for 12 h. The product of first step was purified by flash column chromatography. The product was dissolved in 10 mL trifluoroacetic acid / dichloromethane (1:1, v/v) with stirring for 2 h and the compound 2 obtained by evaporation under reduced pressure. Yield: 99%.

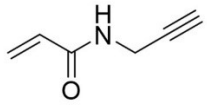


3

Synthesis of compound 3. Eosin Y water soluble (0.65 g, 1.00 mmol) and HATU (0.46 g, 1.20 mmol) were dissolved in 10 mL N,N-Dimethylformamide , and then compound 2(0.31 g, 1.00 mmol) and DIPEA (0.39 g, 3.00 mmol) which dissolved in a little N,N-Dimethylformamide were added dropwise with stirring at room temperature for 24 h. The precipitate was removed by suction filtration and solvent was removed by evaporation under reduced pressure. The above solution was extracted by ethyl acetate three times and then purified by flash column chromatography using acetone/ petroleum ether. Yield: 50%.

Compound 3, ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.78 – 7.62 (m, 3H), 7.57 – 7.47 (m, 1H), 7.04 (d, J = 1.3 Hz, 1H), 5.13 (q, J = 6.7 Hz, 1H), 2.95 (d, J = 43.4 Hz, 1H), 1.61 (p, J = 7.1 Hz, 2H), 1.43 (s, 2H), 1.42 – 1.30 (m, 3H), 0.88 (t, J = 7.3 Hz, 3H).

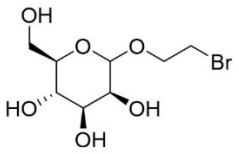
Synthesis of Mannose monomer



4

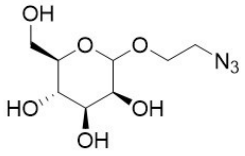
Synthesis of compound 4. Mono-Propargylamine (2.20 g, 39.90 mmol), triethylamine (6.10 g, 60.30 mmol) was dissolved in 20.00 mL dichloromethane. The mixture was cooled to 0 ℃ and acrylylchloride (3.70 g, 40.90 mmol) was added dropwise with stirring for 1 h. The reaction mixture was extracted by water twice and then purified by flash column chromatography using petroleum ether / petroleum ether (2:1, v/v). Yield: 67%.

Compound 4, ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.32 (dd, J = 17.0, 1.4 Hz, 1H), 6.12 (dd, J = 17.0, 10.3 Hz, 2H), 5.68 (dd, J = 10.3, 1.4 Hz, 1H), 4.13 (dd, J = 5.3, 2.6 Hz, 1H), 2.24 (t, J = 2.6 Hz, 1H), 1.82 (d, J = 4.7 Hz, 1H).



5

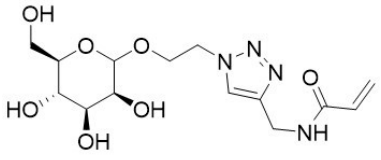
Synthesis of compound 5. D-Mannose (16.60 mmol, 3.00 g) was dissolved in 23.00 mL 2-Bromoethanol and the Silica was added in portions. Concentrated sulfuric acid was added dropwise with stirring at 60℃ for 3 h. Finally, the product was purified by flash column chromatography using ethyl acetate/MeOH (10:1, v/v). Yield: 55%.



6

Synthesis of compound 6. Compound 5 (4.77 g, 16.60 mmol) was dissolved in 20 mL Acetone/water mixture (1:1, v/v) and then the Potassium azide (1.62 g, 19.92 mmol) was added. The solution was stirred at 70 ℃ for 12 h, and then solvent was removed by evaporation under reduced pressure. Finally, the solution was purified by flash column chromatography using Dichloromethane /MeOH (5:1, v/v). Yield: 35%.

^{1}H NMR (400 MHz, Methanol-d_4) δ = 4.85 – 4.80 (m, 1H), 3.99 – 3.91 (m, 1H), 3.90 – 3.82 (m, 2H), 3.74 (td, J = 9.2, 4.6 Hz, 2H), 3.62 (dddt, J = 17.3, 9.6, 5.7, 3.2 Hz, 3H), 3.47 – 3.38 (m, 2H).



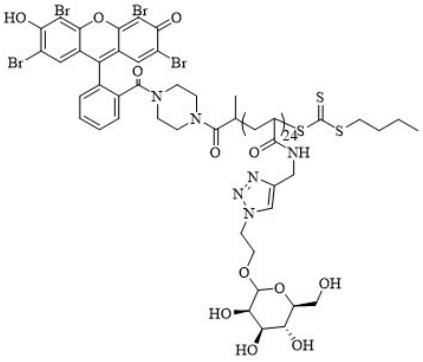
7

Synthesis of compound 7. Compound 6 (0.61 g, 0.24 mmol) and Compound 4 (0.71 g, 0.65 mmol) were dissolved in 5.00 mL methanol. The mixture of L-Ascorbic Acid Sodium Salt (0.64 g, 0.32 mmol) and Copper sulfate

pentahydrate (0.32 g, 0.13 mmol) which dissolved in water (5.00 mL) was added to the above solution with stirring at room temperature for 12 h. Finally, the solution was purified by flash column chromatography using ethyl acetate/MeOH (5:1, v/v). Yield: 89%.

Compound 7, ^{1}H NMR (400 MHz, D_2O) δ = 7.92 (s, 1H), 6.29 - 6.11 (m, 2H), 5.72 (dd, J = 9.8, 1.9 Hz, 1H), 4.04 - 3.97 (m, 1H), 3.88 - 3.81 (m, 1H), 3.78 (dd, J = 3.2, 1.7 Hz, 1H), 3.64 - 3.47 (m, 4H), 2.84 (ddd, J = 8.6, 5.5, 2.3 Hz, 1H).

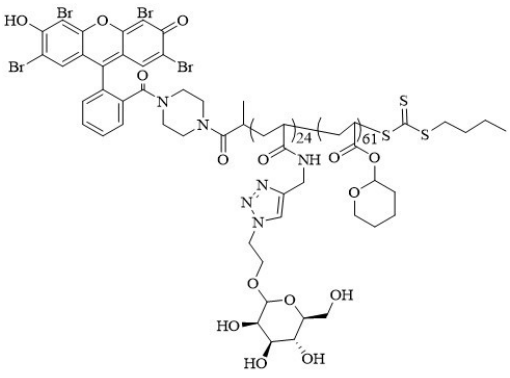
Synthesis of PMAN-RAFT polymers



8

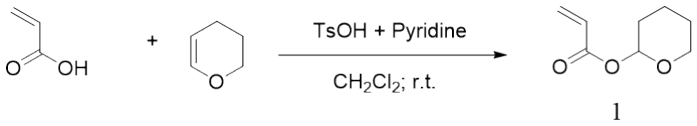
MAN monomer (1.80 g, 5.02 mmol), Eosin Y-RAFT (78.39 mg, 83.72 μmol) and phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide BAPO-ONa（9.99 mg, 27.91 μmol）were dissolved in 6.50 mL of DMSO in 25 mL Schlenk flask with an 8 mm high pressure vacuum valve (SYNTHWARE, China). The reaction solution degassed by bubbling Ar for 30 min and then closed the bottle valve. The reaction solution was stirred at room temperature and irradiated by 365 nm UV curing lights for 3 h. After polymerization, the mixture was purified by dialysis (MWCO:1,000 Da) for 24 h and then obtained by lyophilization.

Synthesis of PMAN-b-PTHPA polymers



9

Polymer 8 (1.00 g, 105.38 μmol), THPA monomer (1.41 g, 9.06 mmol) and BAPO-ONa（12.22 mg, 34.13 μmol）were dissolved in 15.00 mL of DMSO in 50 mL Schlenk flask. The reaction solution degassed by bubbling Ar for 30 min and then closed the bottle valve. The reaction solution was stirred at room temperature and irradiated by illuminated at 365 nm UV curing lights for 5 h. The mixture precipitated three times in cold ether (ice bath) and obtained by Centrifugal at 4000 rpm, 15 min, 4℃.



1

Scheme S1. Synthesis of tetrahydropyranyl acrylate (THPA). TsOH, Pyridine, Dichloromethane, RT, 24 h.

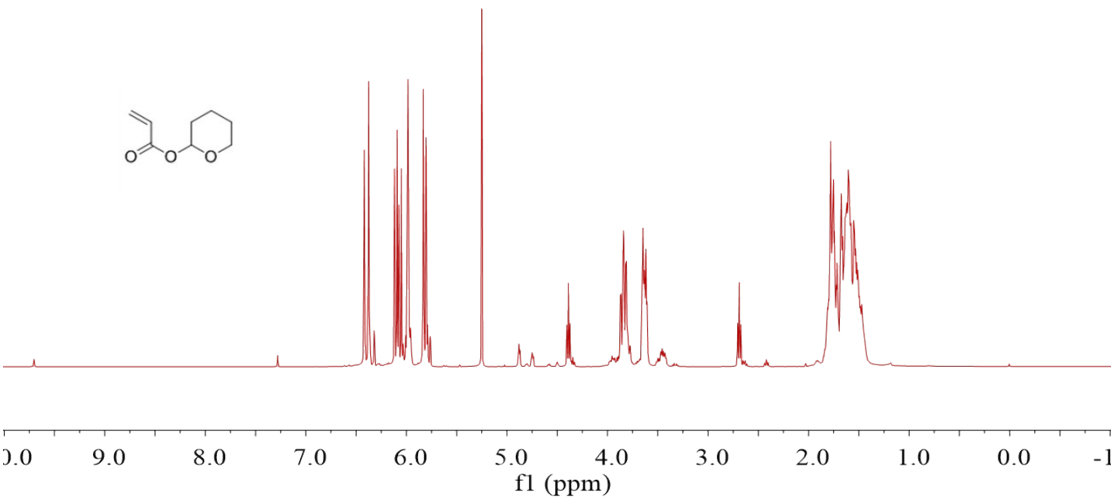
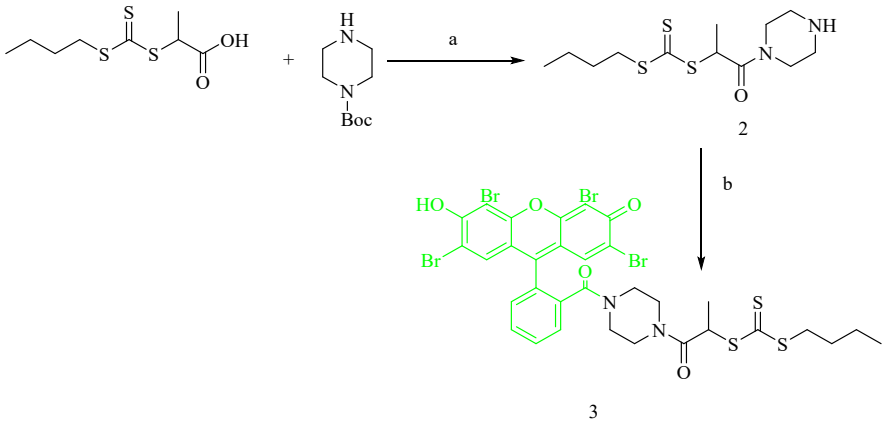


Figure S1. ^{1}H spectrum of THPA recorded in Chloroform-d.



Scheme S2. Synthesis of Green fluorescent CF_3COOH and EosinY-RAFT. a） step 1: DCC (1.1 eq.), DMAP (20 mol%), DCM, rt, 12 h; step 2: CF_3COOH, DCM (1: 1). b） HATU (1.2 eq.), DIPEA (4 eq.), DMF, rt, 24 h.

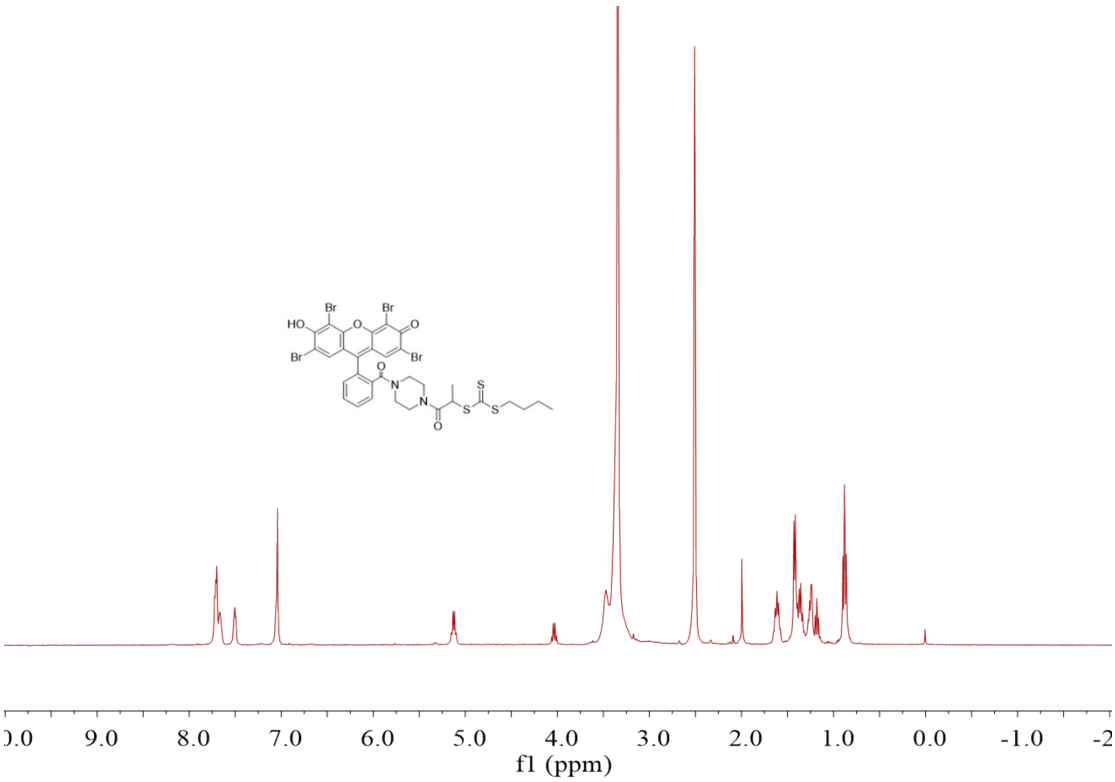


Figure S2. ^1H spectrum of Eosin Y-RAFT recorded in DMSO-d_6.

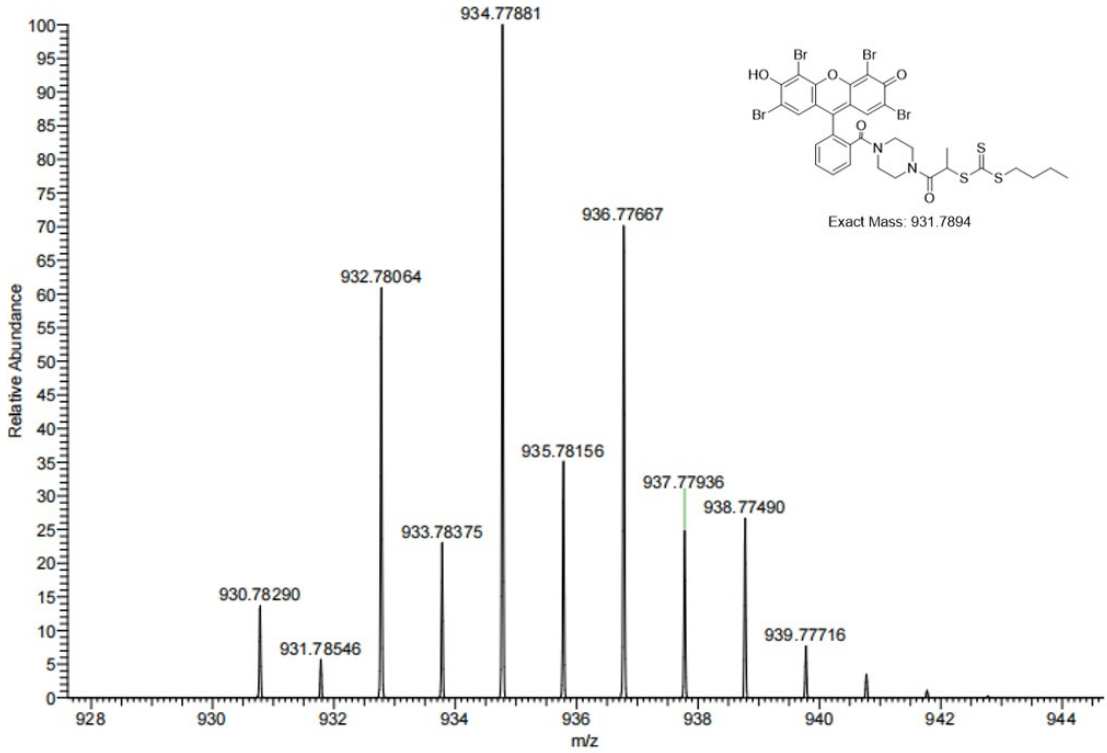
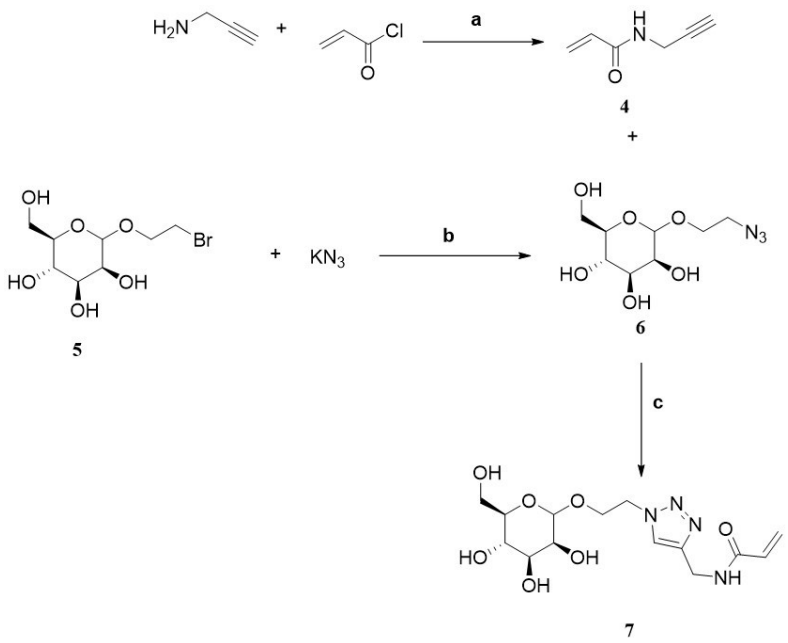


Figure S3. HRMS analysis of compound 3(Eosin Y - RAFT). (ESI) for C32H28Br4N2O5S3 [M+H]-: calcd.: 932.7894; found: 932.78064.



Scheme S3. Synthesis of MAN monomer. a) Et_3N (1.5 eq.), DCM, rt, 1 h. b) H_2O/Acetone, Reflux, 70 ℃, 12 h. c) Vc-Na (0.1 eq.), CuSO4.5H2O (0.05 eq.), MeOH: H_2O = 1:1, rt, 12 h.

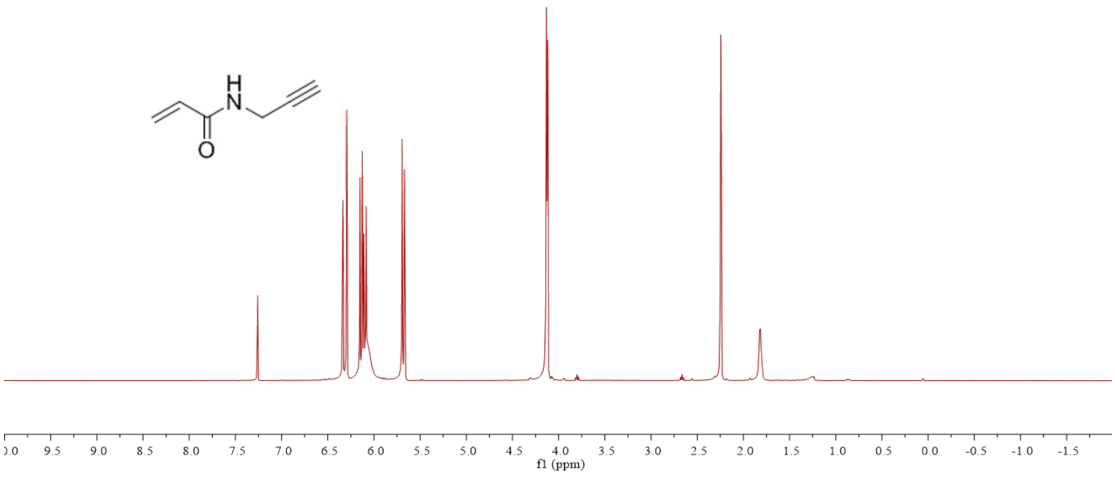


Figure S4. ^1H spectrum of compound 4 recorded in $CDCl_3$.

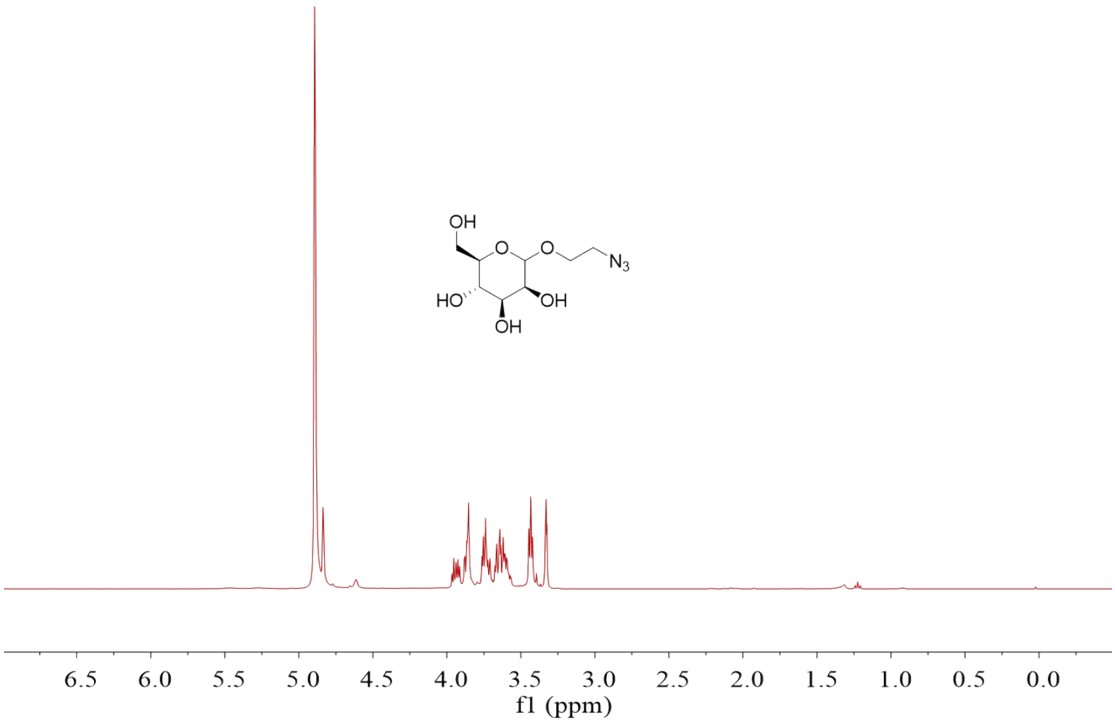


Figure S5. ^{1}H spectrum of compound 6 recorded in $CDCl_3$.

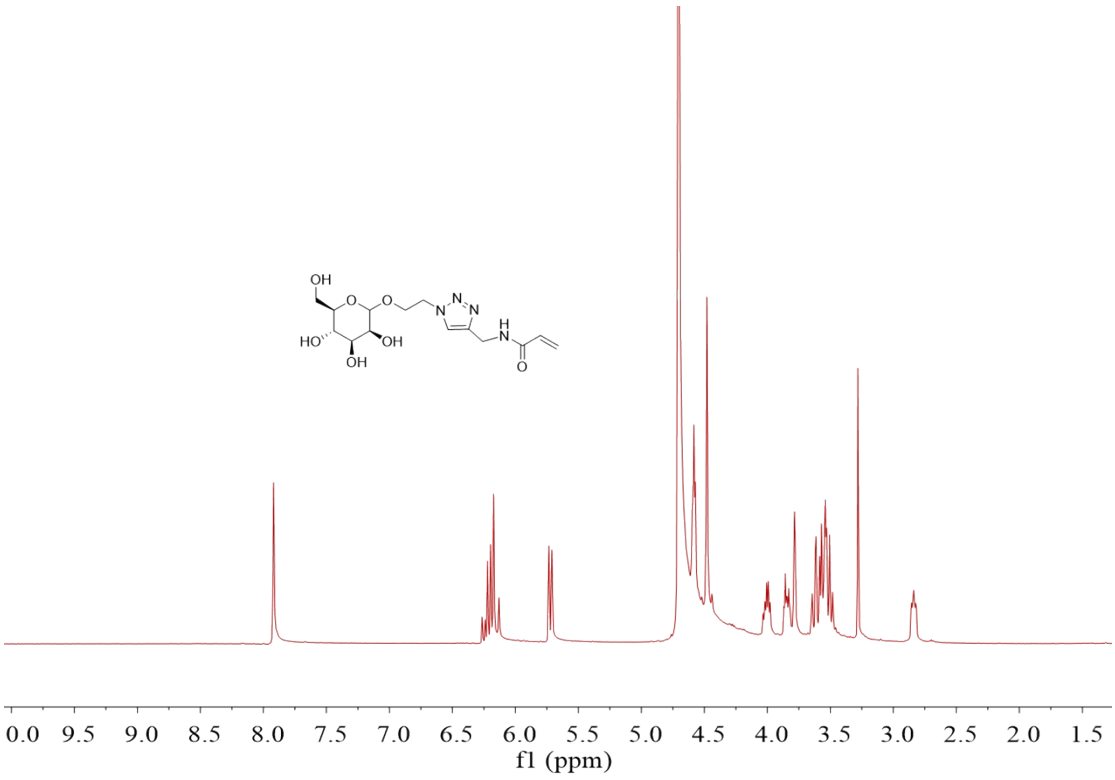
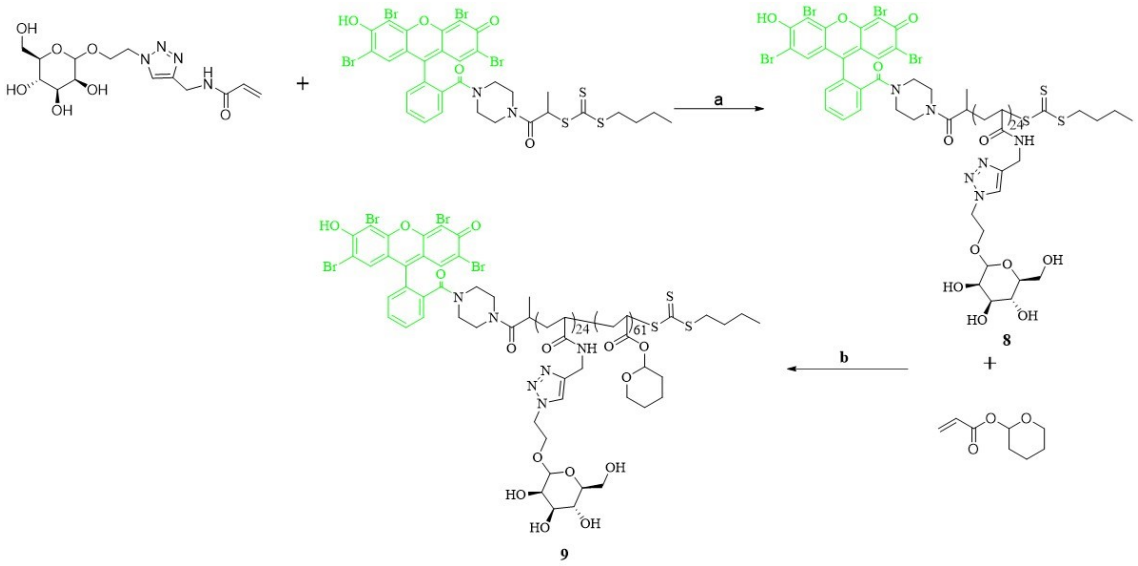


Figure S6. ^{1}H spectrum of compound 7 recorded in D_2O.



Scheme S4. Synthesis of PMAN-*b*-PTHPA polymers. a) BAPO-ONa, DMSO, UV, RT, 3 h. b) polymer 9, THPA monomer, DMSO, 365nm, RT, 5 h. The polymer 8 and polymer 9 were analyzed by ^{1}HNMR respectively.

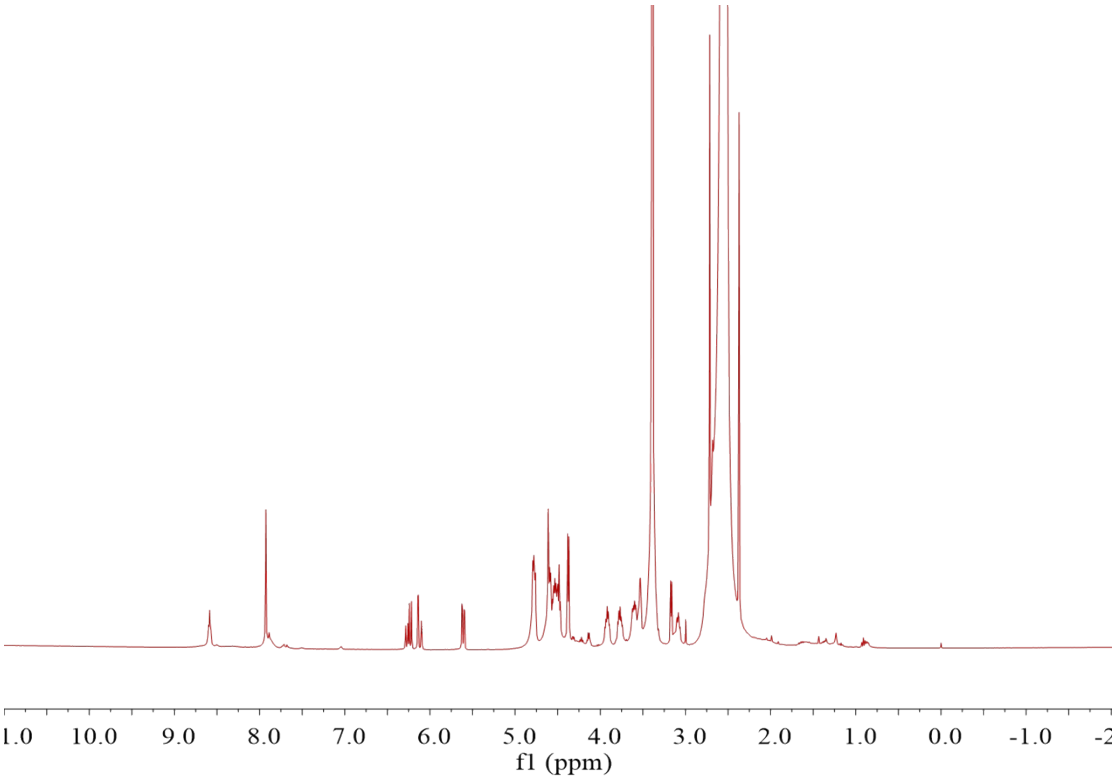


Figure S7. ^{1}H spectrum of polymer 8 reaction mixture after 3 h in DMSO-d_6.

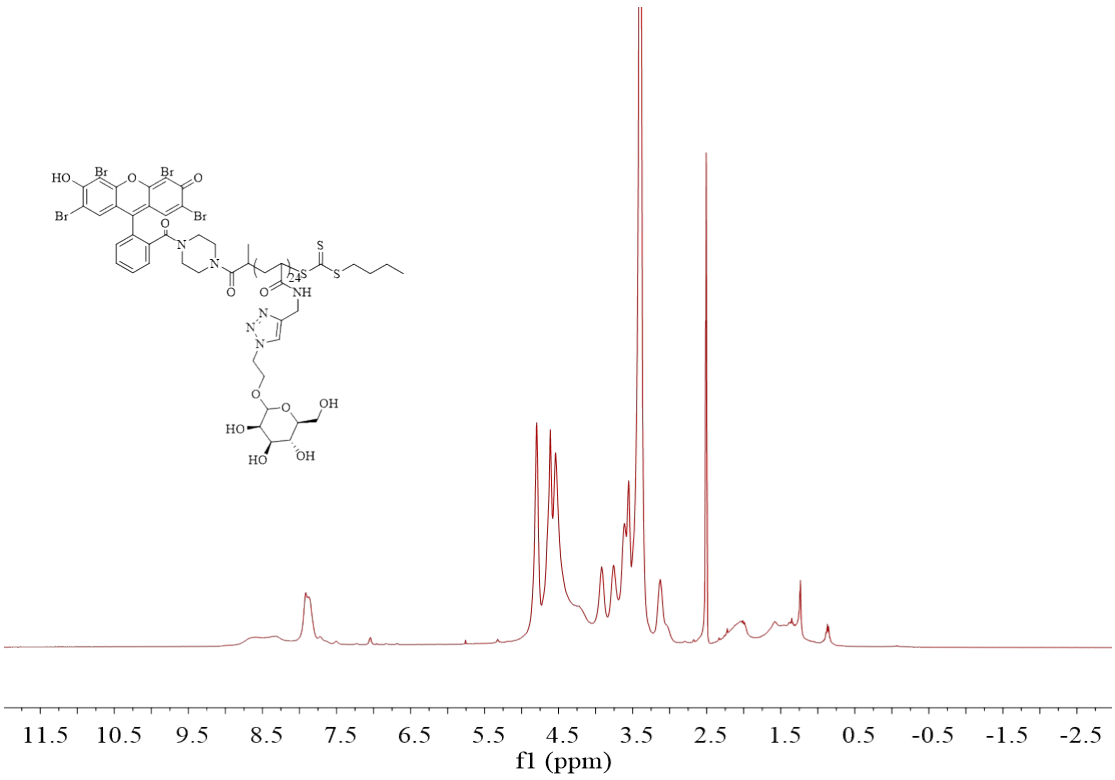


Figure S8. ^{1}H spectrum of polymer 8 recorded in DMSO-d_6.

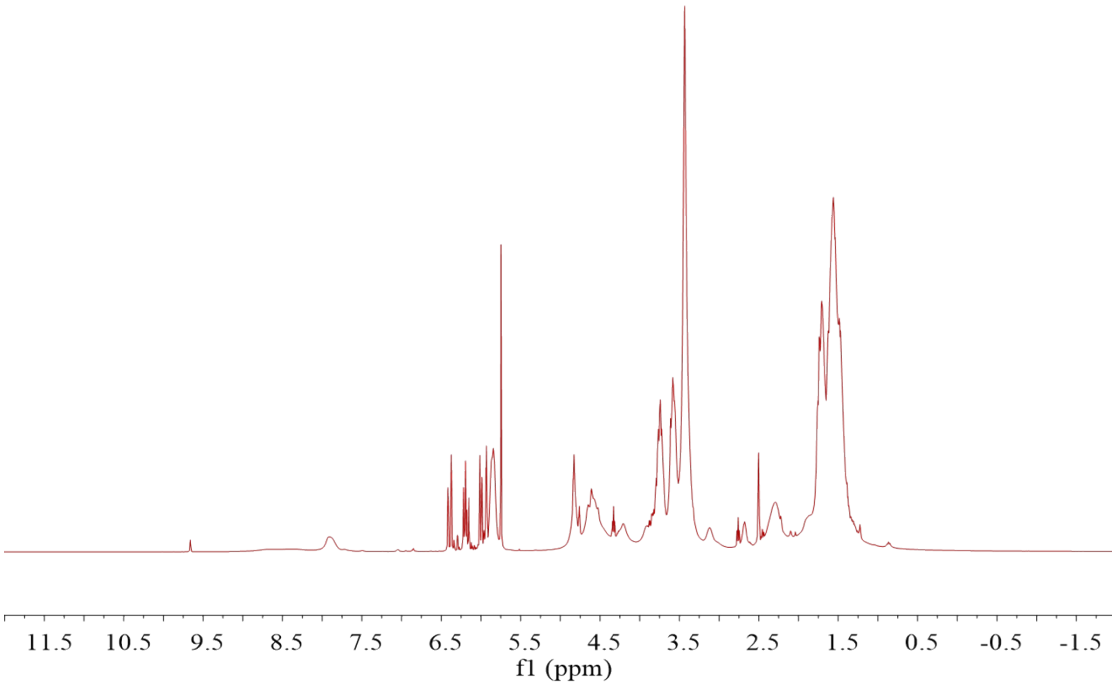


Figure S9. ^{1}H spectrum of polymer 9 reaction mixture after 5 h in DMSO-d_6.

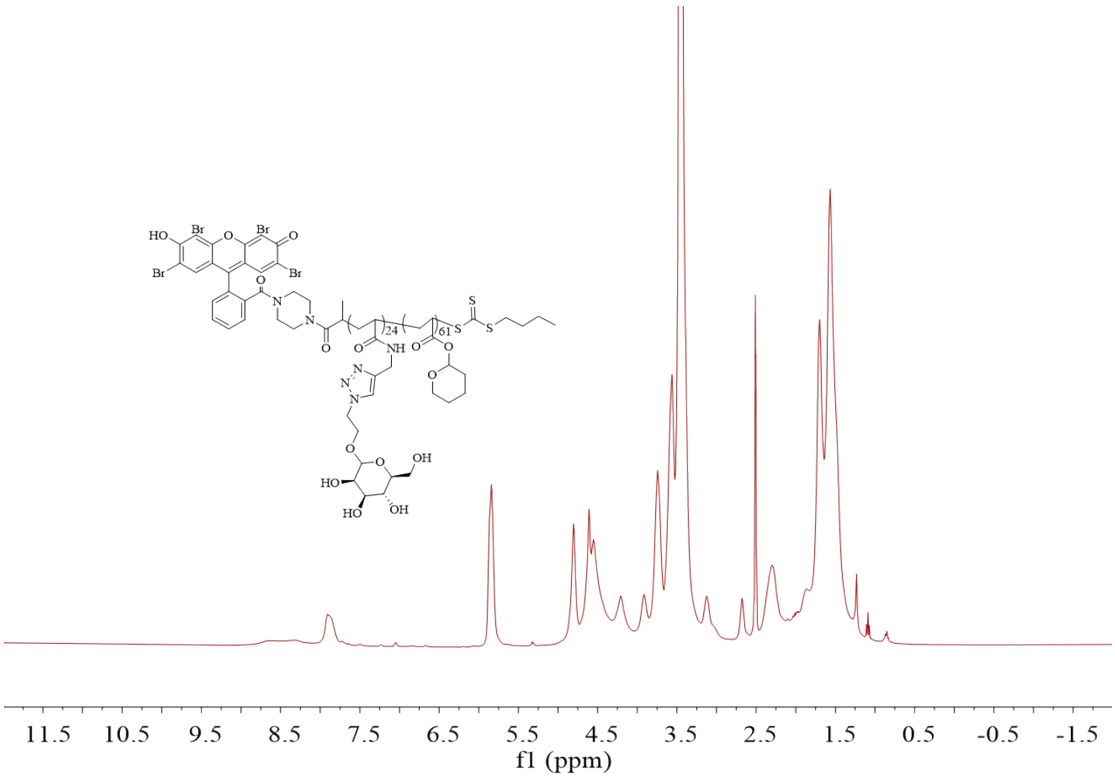


Figure S10. ^{1}H spectrum of polymer 9 recorded in DMSO-d_6.

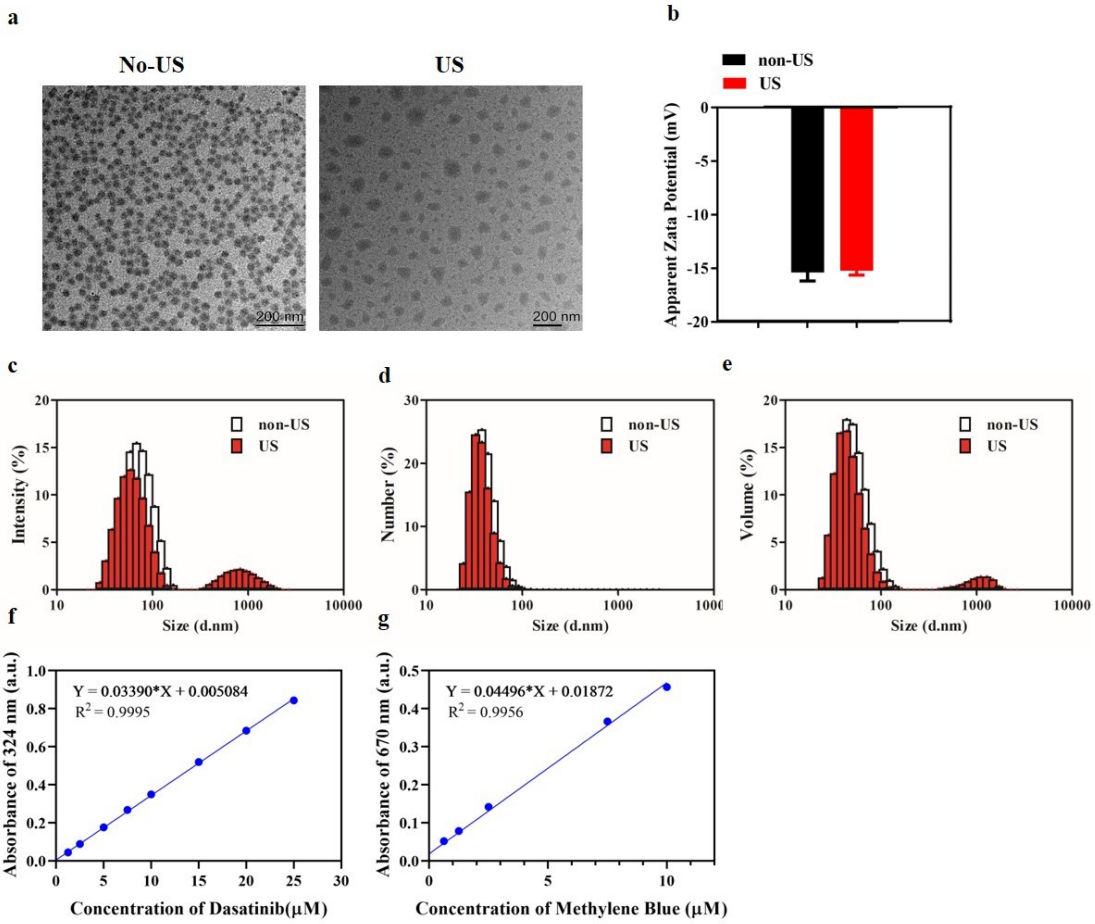


Figure S11. a) TEM images of DAS/MB@M before and after ultrasound irradiation. b), c) d) and e) The Zeta potential and size distribution of DAS/MB@M before and after ultrasound irradiation in water. f) and g) The Standard curve of Dasatinib and Methylene Blue with absorbance at 324 nm and 670 nm respectively.

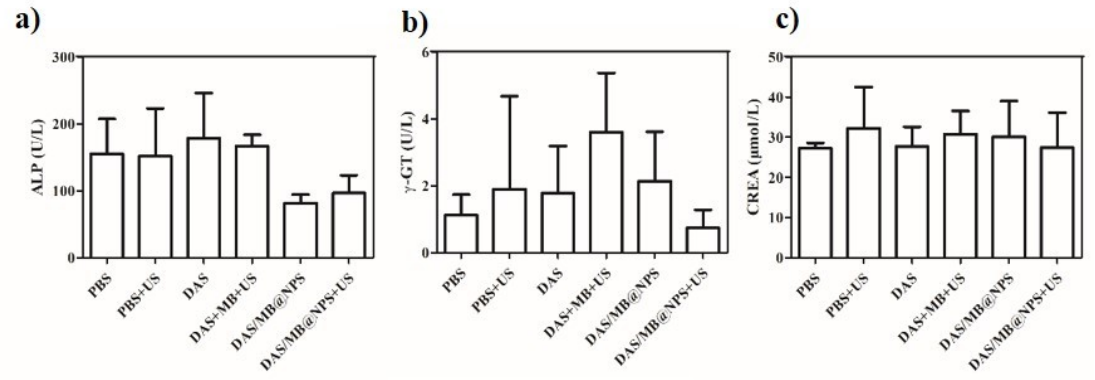


Figure S12. a-c) Blood biochemistry analysis (alanine aminotransferase (ALT), γ-GT (γ-glutamyl transpeptidase) and creatinine (CREA).

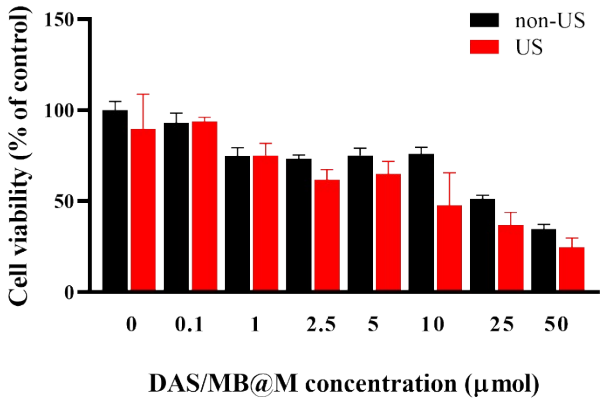


Figure S13. HepG2 cell viability against DAS/MB@M under US irradiation.

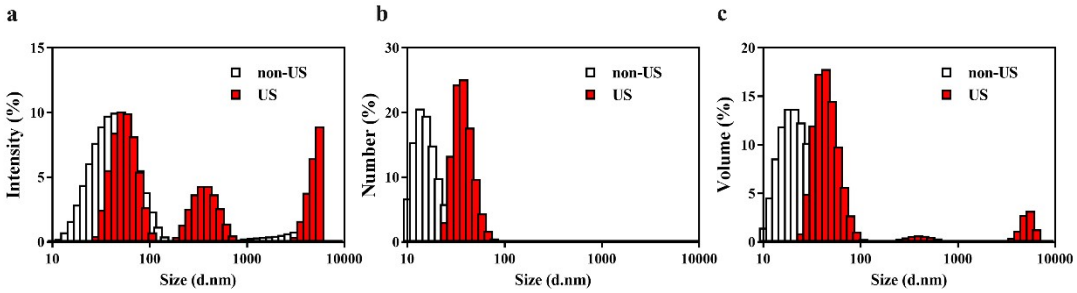


Figure S14. The DLS diagrams change of DAS/MB@M before and after ultrasound irradiation in water (0.25 MHz, 1.7W/cm−2, 20%, 15min) based on a) Intensity%, b) Number% and c) Volume%.

Reference

1. J. Geng, W. Li, Y. Zhang, N. Thottappillil, J. Clavadetscher, A. Lilienkampf and M. Bradley, *Nature Chemistry*, 2019, **11**, 578-586.

2. J.-T. Lee, M. C. George, J. S. Moore and P. V. Braun, *Journal of the American Chemical Society*, 2009, **131**, 11294-11295.